

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Serial No.: 09/328,975 )

Filed: 6/9/99 )

Group Art Unit: 1632 )

Examiner: Richard Schnizer

For: Charge Reversal of Polyion Complexes

DECLARATION UNDER 37 C.F.R. §1.132

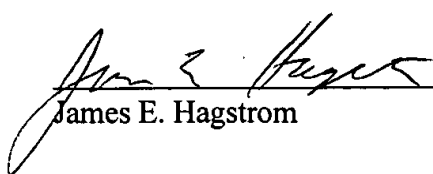
Assistant Commissioner for Patents  
Washington, DC 20231

Dear Sir:

I, James E. Hagstrom, hereby declare as follows:

1. I am an inventor of the captioned application.
2. Applicants' process was conceived prior to the effective date of the Office Action prior art references.
3. We developed our recharging process with due diligence from conception to the filing of our application.
4. Photocopies of my personal laboratory notebook pages showing nucleic acids recharged with histone and then re-recharged with liposomes dated March and June, 1994 accompany this Declaration.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

 9/7/01  
James E. Hagstrom Date

3/28/

## RAT Myotube Transfection

↓ ALL DNA in pH 8.5 Tris

- <sup>2</sup>> 5 ug pSALUX 5ug/plate  
<sup>3</sup>> DNA + Lipofectin 2.5 $\lambda$ /plate  
<sup>5</sup>> DNA + Lipofectin 15 $\lambda$ /plate  
<sup>7</sup>> DNA + Lipo (2.5 $\lambda$ /plate) + NLS-H1 (0.5 $\lambda$ /plate)<sup>(1.2 $\lambda$ )</sup>  
<sup>9</sup>> DNA + Lipo (2.5 $\lambda$ /plate) + NLS-H1 (1.5 $\lambda$ /plate)<sup>(3.4 $\lambda$ )</sup>  
<sup>11</sup>> DNA + Lipo (2.5 $\lambda$ /plate) + NLS-H1 (0.5 $\lambda$ /plate)<sup>(1.2 $\lambda$ )</sup> + MAT-H1 (0.5 $\lambda$ /plate)<sup>(0.5 $\lambda$ )</sup>  
<sup>13</sup>> DNA + Lipo (2.5 $\lambda$ /plate) + NLS-H1 (0.5 $\lambda$ /plate)<sup>(1.2 $\lambda$ )</sup> + MAT-H1 (1.5 $\lambda$ /plate)<sup>(6.8 $\lambda$ )</sup>  
<sup>15</sup>> DNA + Lipo (2.5 $\lambda$ /plate) + NLS-H1 (1.5 $\lambda$ /plate)<sup>(3.4 $\lambda$ )</sup> + MAT-H1 (0.5 $\lambda$ /plate)<sup>(0.5 $\lambda$ )</sup>  
<sup>17</sup>> DNA + Lipo " + NLS-H1 (1.5 $\lambda$ /plate)<sup>(3.4 $\lambda$ )</sup> + MAT-H1 (1.5 $\lambda$ /plate)<sup>(6.8 $\lambda$ )</sup>  
<sup>19</sup>> DNA + " " + NLS-H1 3 $\lambda$ /plate<sup>(6.8 $\lambda$ )</sup>  
<sup>21</sup>> DNA + Lipo " + NLS-H1 4.5 $\lambda$ /plate<sup>(10.2 $\lambda$ )</sup>  
<sup>23</sup>> [DNA + NLS-H1 (1.5 $\lambda$ )<sup>(3.4 $\lambda$ )</sup>] + DOPE (6 $\lambda$ /plate)<sup>(1.2 $\lambda$ )</sup>  
<sup>25</sup>> [DNA + NLS-H1 (1.5 $\lambda$ )<sup>(3.4 $\lambda$ )</sup> + MAT-H1 (0.5 $\lambda$ )<sup>(0.5 $\lambda$ )</sup>] + DOPE (6 $\lambda$ /plate)<sup>(1.2 $\lambda$ )</sup>  
<sup>27</sup>> [DOPE + NLS-H1 (1.5 $\lambda$ )<sup>(3.4 $\lambda$ )</sup>] + DNA (5 $\lambda$ /plate)<sup>(1.2 $\lambda$ )</sup>  
<sup>29</sup>> [DOPE + NLS-H1 (1.5 $\lambda$ )<sup>(3.4 $\lambda$ )</sup> + MAT-H1 (0.5 $\lambda$ )<sup>(0.5 $\lambda$ )</sup>] + DNA  
<sup>31</sup>> DNA 5 $\lambda$ /plate + DOPE 6 $\lambda$ /plate

## 10 Day Old Myotubes -

- <sup>29</sup>> DNA (5 $\lambda$ /plate) + Lipo (2.5 $\lambda$ /plate)  
<sup>39</sup>> DNA " + " " + NLS-H1 (1.5 $\lambda$ /plate)<sup>(3.4 $\lambda$ )</sup>  
<sup>59</sup>> DNA " + Lipo " + NLS-H1 " + MAT-H1 (0.5 $\lambda$ )<sup>(0.5 $\lambda$ )</sup>

† For all samples except 23-32  
 - Add DNA (10 $\mu$ g/2 plates) in 100 $\mu$ l Tris 8.5 + Protein  
     ↓ 15' rt  
     Add Lipofectin  
     ↓ 80' rt

↓  
Add to 1.5 ml on each 35mm dish

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5:28

MEAS. TIME (s) 31.0

SAMPLE	RLU	
1	543	H <sub>2</sub> O. BIK
2	9204	11 Lucif.
3	87503	51 Lucif.
4	1436	> 5 ug/plate pBSRLUX
5	2385	
6	44422	> DNA + 2.5 $\mu$ /plate Lipofectin
7	28742	
8	227053	> DNA + Lipo.
9	415636	1.5 $\mu$ /plate
10	15875	> DNA + Lipo + NLS-H1 (0.5 $\mu$ /plate)
11	7301	(2.5)
12	75306	> DNA + Lipo + NLS-H1 + MAT-H1
13	727847	(1.5 $\mu$ /plate)
14	55092	> DNA + Lipo + NLS-H1 + MAT-H1
15	18582	0.5 $\mu$ /plate (0.5 $\mu$ /plate) CB
16	41314	> DNA + Lipo + NLS-H1 + MAT-H1
17	12334	0.5 $\mu$ (1.5 $\mu$ /plate) CB
18	18130	> DNA + Lipo + NLS-H1 + MAT-H1 (0.5 $\mu$ )
19	31812	(1.5 $\mu$ ) CB
20	40416	> DNA + Lipo + NLS-H1 + MAT-H1
21	30253	(1.5 $\mu$ ) (1.5 $\mu$ ) CB
22	2381512	> DNA + Lipo + NLS-H1
23	939243	(2 $\mu$ /plate)
24	5892126	> DNA + Lipo + NLS-H1
25	3102207	4.5 $\mu$ /plate
26	182370	[DNA + NLS-H1] + DOPE 60 $\mu$ /plate
27	331964	1.5 $\mu$
28	225842	[DNA + NLS-H1 + MAT-H1] + DOPE
29	248196	(1.5 $\mu$ ) (0.5 $\mu$ ) CB
30	132754	[DOPE + NLS-H1] + DNA
31	119066	(1.5 $\mu$ )
32	406366	[DOPE + NLS-H1 + MAT-H1] + DNA
33	240774	1.5 $\mu$ CB
34	97183	[DNA + DOPE] 150 $\mu$ /plate
35	18896	

\* MAT-H1 - purified via  
Ni-NTA agarose and then  
Cibacron Blue agarose

PC... loaded onto CB column  
at 150mM NaCl + eluted  
off with 500mM increasing  
NaCl step gradient  
- checked on 12% SDS-PAGE  
- concentrated single band  
containing (MAT-H1) elution  
- contained 10  
- assayed protein (conc)

- Results - Appears that  
CB purified protein  
inhibits transfectability

\* Does it bind & retard  
DNA in a gel-shift assay

6/7/94.

## PS/PE expt #2

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1	>	PBSRGYcat <sup>(3ug)</sup>	+	PS 2.5 <sup>18λ</sup>	+	NLS-H1 <sup>(2ug)</sup>	8λ	(300 DNA Prot)	+	(300 Liposomes)
2	>	DNA	+	PS 2.5 <sup>18λ</sup>	+	"	(4ug)	16λ		
3	>	DNA	+	PS 2.5 <sup>18λ</sup>	+	NLS H1	(6ug)	24λ		
4	>	DNA	+	PS 5 <sup>18λ</sup>	+	"	(2ug)	8λ		
5	>	DNA	+	PS 5 <sup>18λ</sup>	+	"	(4ug)	16λ		
6	>	DNA	+	PS 5 <sup>18λ</sup>	+	"	(6ug)	24λ		
7	>	DNA	+	PS 10 <sup>18λ</sup>	+	"	(2ug)	8λ		
8	>	DNA	+	PS 10 <sup>18λ</sup>	+	"	(4ug)	16λ		
9	>	DNA	+	PS 10 <sup>18λ</sup>	+	"	(6ug)	24λ		
10	>	DNA	+	PS 2.5 <sup>36λ</sup>	+	"	(4ug)	16λ		
11	>	DNA	+	PS 2.5 <sup>36λ</sup>	+	"	(6ug)	24λ		
12	>	DNA	+	PS 5 <sup>36λ</sup>	+	"	(4ug)	16λ		
13	>	DNA	+	PS 5 <sup>36λ</sup>	+	"	(6ug)	24λ		
14	>	DNA	+	PS 10 <sup>36λ</sup>	+	"	(4ug)	16λ		
15	>	DNA	+	PS 10 <sup>36λ</sup>	+	"	(6ug)	24λ		
16	>	DNA	+	PS 2.5 <sup>72λ</sup>	+	"	(4ug)	16λ		
17	>	DNA	+	PS 2.5 <sup>72λ</sup>	+	"	(6ug)	24λ		
18	>	DNA	+	PS 5 <sup>72λ</sup>	+	"	(4ug)	16λ		
19	>	DNA	+	PS 5 <sup>72λ</sup>	+	"	(6ug)	24λ		
20	>	DNA	+	PS 10 <sup>72λ</sup>	+	"	(4ug)	16λ		
21	>	DNA	+	PS 10 <sup>72λ</sup>	+	"	(6ug)	24λ		
22	>	DNA	+	<del>PS 2.5</del>	+	"	(4ug)	16λ		
23	>	DNA	+	<del>PS 5</del>	+	"	(6ug)	24λ		
24	>	DNA	+	<del>PS 10</del>	+	"	(4ug)	16λ		
25	>	DNA	+	<del>PS 2.5</del>	+	"	(6ug)	24λ		
26	>	DNA	+	<del>PS 5</del>	+	"	(4ug)	16λ		
27	>	DNA	+	<del>PS 10</del>	+	"	(6ug)	24λ		
28	>	DNA	+	<del>PS 2.5</del>	+	"	(4ug)	16λ		
29	>	DNA	+	<del>PS 5</del>	+	"	(6ug)	24λ		
30	>	DNA	+	<del>PS 10</del>	+	"	(4ug)	16λ		
31	>	DNA	+	<del>PS 2.5</del>	+	"	(6ug)	24λ		
32	>	DNA	+	<del>PS 5</del>	+	"	(4ug)	16λ		
33	>	DNA	+	<del>PS 10</del>	+	"	(6ug)	24λ		
34	>	DNA	+	<del>PS 2.5</del>	+	"	(4ug)	16λ		
35	>	DNA	+	<del>PS 5</del>	+	"	(6ug)	24λ		
36	>	DNA	+	<del>PS 10</del>	+	"	(4ug)	16λ		

## Protocol

- Add DNA + protein (300λ optimum) 15' at rt
- Add Liposomes in (300λ optimum) 15' at rt
- WASH cells 1X in optimum
- Add complex to 2ml optimum on cells
- change media after 3-4 hrs
- incubate at 37°C for ~ 48 hrs
- Harvest cells
- lux assay

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1	1191	RLU			
2	466		> DNA + PS 2.5 + NLS-H1	(18λ)	(2ug)
3	66525		> DNA + PS 2.5 + NLS-H1	18λ	4ug
4	107400				
5	2181385		> DNA + PS 2.5 + NLS-H1	18λ	(6ug)
6	1997461				
7	485		> DNA + PS 5 + NLS-H1	18λ	(2ug)
8	487				
9	843961		> DNA + PS 5 + NLS-H1	18λ	(4ug)
10	799857				
11	2443514		> DNA + PS 5 + NLS-H1	18λ	(6ug)
12	1974928				
13	686		> DNA + PS 10 + NLS-H1	18λ	(2ug)
14	665				
15	42442		> DNA + PS 10 + NLS-H1	18λ	(4ug)
16	31953				
17	1930695		> DNA + PS 10 + NLS-H1	18λ	(6ug)
18	1779858				
19	366265		> DNA + PS 2.5 + NLS-H1	(36λ)	(4ug)
20	649356				
21	2776187		> DNA + PS 2.5 + NLS-H1	(36λ)	(4ug)
22	1149081				
23	1596987		> DNA + PS 5 + NLS-H1	(36λ)	(4ug)
24	1792688				
25	2789949		> DNA + PS 5 + NLS-H1	(36λ)	(6ug)
26	3353918				
27	270468		> DNA + PS 10 + NLS-H1	(36λ)	(4ug)
28	236696				
29	2482591		> DNA + PS 10 + NLS-H1	(36λ)	(6ug)
30	2774275				
31	2890371		> DNA + PS 10 + NLS-H1	(36λ)	(9ug)
32	2966735				
33	273358		> DNA + Lipofectin		
34	285150				
35			> DNA + Lipofectin		